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ORIGINAL PAPER

The ectomycorrhizal fungus *Tricholoma matsutake* is a facultative saprotroph in vitro

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Abstract *Tricholoma matsutake* is an economically important ectomycorrhizal fungus of coniferous woodlands. Mycologists suspect that this fungus is also capable of saprotrophic feeding. In order to evaluate this hypothesis, enzyme and chemical assays were performed in the field and laboratory. From a natural population of *T. matsutake* in southern Finland, samples of soil–mycelium aggregate (shiro) were taken from sites of sporocarp formation and nearby control (PCR-negative) spots. Soil organic carbon and activity rates of hemicellulolytic enzymes were measured. The productivity of *T. matsutake* was related to the amount of utilizable organic carbon in the shiro, where the activity of xylosidase was significantly higher than in the control sample. In the laboratory, sterile pieces of bark from the roots of Scots pine were inoculated with *T. matsutake* and the activity rates of two hemicellulolytic enzymes (xylosidase and glucuronidase) were assayed. Furthermore, a liquid culture system showed how *T. matsutake* can utilize hemicellulose as its sole carbon source. Results linked and quantified the general relationship between enzymes secreted by *T. matsutake* and the degradation of hemicellulose. Our findings suggest that *T. matsutake* lives mainly as an ectomycorrhizal symbiont but can also feed as a saprotroph. A flexible trophic ecology confers *T. matsutake* with a clear advantage in a heterogeneous environment and during sporocarp formation.

Keywords Fungal ecology · Hemicellulose · Saprotrophic · Shiro · *Tricholoma matsutake* · Xylosidase

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Introduction

The conventional view of ectomycorrhizal (ECM) fungi is that they depend on their plant hosts for a supply of photosynthetic carbon. However, the extent to which ECM fungi are constrained to their symbiotic lifestyle was recently questioned (Taylor and Alexander 2005) and the debate currently focuses on the evidence concerning their facultative saprotrophy (Baldrian 2009; Cullings and Courty 2009). Partly due to this debate, more attention has been paid to the diversity of ways in which ECM fungi acquire carbon in natural ecosystems (Courty et al. 2010; Talbot et al. 2008).

Tricholoma matsutake (S. Ito et Imai) is an ectomycorrhizal fungus that forms a symbiotic association with *Pinus* and *Picea* in the Northern Hemisphere (Hosford et al. 1997;

Yamada et al. 1999; Vaario et al. 2010a). It produces mushrooms that are commercially important in Japan (Suzuki 2005) and the increasing value of this non-woody forest product has been noticed in Nordic countries, where it was known as *Tricholoma nauseosum* until recently when molecular techniques revealed its conspecificity with *T. matsutake* (Bergius and Danell 2000). Over the past 50–60 years, the increasing rarity of this edible mushroom in Japan has stimulated research into improving sporocarp formation in nature (e.g., by outplanting mycorrhizal seedlings) and cultivating matsutake under controlled conditions. However, results have not been encouraging and artificial culture of matsutake remains very limited. However, a better understanding of matsutake ecology and sporocarp formation might offer a solution.

Matsutake exists in the shiro, a unique and massive aggregate of mycorrhizal mycelia, host plant roots, and soil particles (Hosford et al. 1997; Ogawa 1978). Based on field observations, *T. matsutake* typically colonizes pine or spruce root tips, which are often necrotic and dead-looking during the spring season (Ogawa 1975; Gill et al. 2000; Vaario et al. unpublished); however, the fungus can also inhabit organic material such as bark pieces (Vaario et al. 2002), which suggests that it is capable of acquiring carbon independently of a host plant. The bark of Scots pine contains 44.9% Klason lignin, 25.4% cellulose, and 14.7% hemicellulose (Valentin et al. 2009). Matsutake is known to produce the cellulolytic enzyme β-glucosidase (Vaario et al. 2002; Kusuda et al. 2006), but evidence of cellulose degradation has yet to be observed. Hemicelluloses are a relatively water-soluble source of organic carbon that can be leached to lower soil layers where the shiro is found. Though it seems likely, the ability of *T. matsutake* to utilize hemicellulose has yet to be demonstrated.

The objectives of this study are to evaluate the extent to which *T. matsutake* can feed saprotrophically on hemicellulotic carbohydrates. We measured enzyme activity and growth on several organic carbon substrates under controlled conditions and investigated enzyme activity and profiled hemicellulose concentration in soil dominated by *T. matsutake*. We tested the following hypotheses: (1) *T. matsutake* can exist and grow as a saprotroph and (2) the productivity of sporocarps is related to the amount of available organic carbon in the shiro.

Materials and methods

Experimental design

Our study measured enzyme activity rates in the field and laboratory and related them to soil chemical properties (field) and organic carbon source (laboratory). In the field component, soil enzyme activity and organic carbon properties were linked with the productivity of *T. matsutake*. The laboratory phase consisted of two experiments: (1) Petri dish microcosms enabled enzyme activities and fungal growth to be accurately measured and (2) liquid media containing pre-determined carbon sources were maintained in glass flasks to observe and quantify any saprophytic feeding of *T. matsutake*.

Study area

The field study was conducted in the Nuuksio national park (60°18′16″ N, 24°31′10″ E) in southern Finland. We surveyed a 100×135-m (0.0135 km^2) area that contained sporocarps of *T. matsutake* in 2007. Within the study area, there were 37 *Pinus sylvestris*, 7 *Picea abies*, and 8 *Betula pendula* trees taller than 2 m. Sporocarps were recorded continuously during 2008–2010 (Fig. 1). Based on the distribution of sporocarps, we estimated there to be five separate patches of *T. matsutake* fruiting sites in the study area. Within these five patches, 67 and 39 sporocarps were harvested in 2009 and 2010, respectively. According to their productivity during 2009 and 2010, patches 1 and 2 were designated as of low productivity (L: lower than average) and patches 3–5 were designated as of high productivity (H: higher than average) (Fig. 1). Soil chemical properties were determined after sporocarps were harvested in 2009. Soil moisture was determined by drying at 105°C overnight and organic matter content was determined as loss-on-ignition after combustion at 550°C for 4 h. Carbon and nitrogen were determined from air-dried soil with a LECO CHN-1000 analyser according to ISO 10694 and ISO 13878. Soil chemical properties and sporocarp numbers 2009–2010 are described in Table 1.

Laboratory work

Petri dish microcosm

Pieces of root bark were collected from a fallen *P. sylvestris* 500 m south of the study site and ground with a IKA® MF10 Mill (sieve size diameter, 2 mm) (Staufen, Germany). Bark pieces less than 2 mm^2 were collected and autoclaved twice at 121°C for 20 min with a 3-day interval. Glass beads (diameter 3 mm) (Lenz, Laborglas GmbH & Co. KG, Germany) were used as a control substrate. A total of 500 mg of autoclaved bark pieces or glass beads was spread onto the surface of 15 ml water agar medium (1.2%) in a Petri dish. To moisten the system, 0.5 ml sterilized water was added to the bark pieces or glass beads. The Petri dishes were then left on a clean bench while the bark pieces absorbed the water. Three 6-mm plugs were cut from the actively growing margin of a *T. matsutake* colony cultured on modified MMN medium and placed inverted on the bark pieces or glass beads. Each isolate inoculated three plates

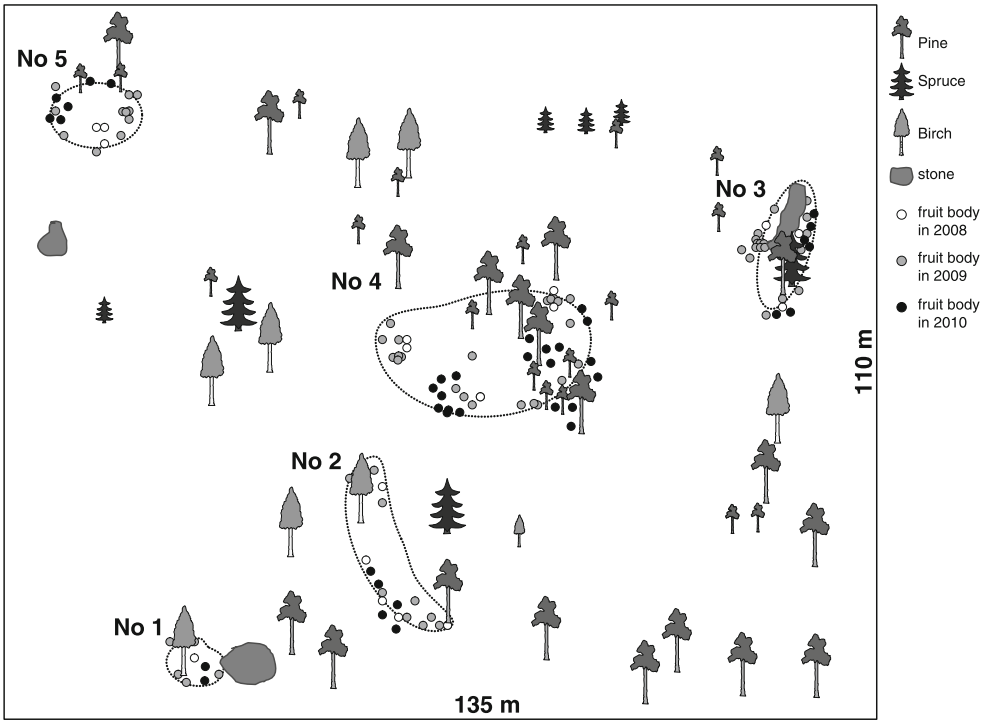

Fig. 1 Map of the study area in a national park in southern Finland

(replicates). The bark–water agar plate without *T. matsutake* inoculation served as a negative control. Cultures were grown in the dark at room temperature. Hyphal growth was observed under a dissecting microscope. At 20 and 40 days after inoculation, five bark pieces were taken immediately below each inoculation plug and combined prior to the hemicellulolytic enzyme assay. In total, nine samples per isolate were measured on each date. The quantification of hemicellulotic compounds (as the sum of each carbohydrate in bark pieces prior to inoculation) is described below.

Glass flask culture

A basic medium (BM) modified from MMN (Marx 1969) contained $NH_4H_2PO_4$ 0.25 g, KH_2PO_4 0.5 g, $CaCl_2$ 0.05 g, NaCl 0.025 g, $MgSO_4{\cdot}7H_2O$ 0.15 g, FeEDTA 0.0168 g, and thiamine HCl 0.01 g/l. Two types of hemicellulose and one type of cellulose were added separately as sole carbon source in the medium. Hemicellulose products were prepared as in Leppänen et al. (2011): one from spruce (Hs) and the other from birch (Hb). We used a commercial source of cellulose (Cel) obtained from spruce (Fluka Chemie AG, product no. 22182). BM containing glucose was used as a carbon positive control (C+). Carbon-containing media contain 1 gl^{-1} carbon source, Celg medium contains additional 0.1 gl^{-1} glucose. BM without added carbon was used as a negative control (C−). All media were adjusted to pH 5.5 prior to autoclaving. Three mycelial plugs were taken as described above and placed into a flask containing 30 ml liquid medium. Three plugs per isolate were stored at −20°C for dry weight measurement at day 0. All cultures were maintained in the dark at

Table 1 Number of matsutake sporocarps and mean values of soil chemical properties at the study site

Samples	Sporocarps		OM,%		C,%		N,%		Soil moisture,%		Cellulose (mg g^{-1} dw soil)	
	2009	2010										
HShiro+abv	>Average	>Average	63.7	a[a]	42.5	a	1.4	a	4.0	a	72.8±4.0	a
LShiro+abv	<Average	<Average	54.7	ab	33.0	ab	1.1	a	3.4	a	48.3±13.1	b
Shiro-abv	0	0	46.0	b	28.0	b	0.8	b	3.2	a	na	
Hshiro+	>Average	>Average	12.9	a′	6.7	a′	0.2	a′	1.3	a′	9.5±1.6	a′
LShiro+	<Average	<Average	8.0	a′b′	4.0	a′b′	0.1	a′	0.9	b′	5.1±1.5	a′
Shiro−	0	0	7.5	b′	3.8	b′	0.1	a′	0.7	b′	na	

[a] The mean (±SEs) difference is significant at the 0.05 level, n=6–15 (LSD)

room temperature. At the start of the experiment and 12 days after inoculation, 2 ml of media was sampled and stored at −20°C for hemicellulotic sugar analysis. After 2 months of incubation, cultures were collected with glass microfiber filter disc (GFB 47 mm in diameter, Whatman®) and pre-weighed after 12 h of drying at 75°C. All filtrate on the glass microfiber filter disc was dried at 75°C for 12 h. Net increase in biomass (growth) was calculated from final minus initial mycelium dry weights. A total of three cultures per isolate was measured for growth in liquid media.

Fungal isolates

Three isolates of *T. matsutake* (S. Ito et Imai) Sing. were used in the plate and flask experiments: Japanese isolate (JA: Tm 0945, Matsushita et al. 2005), Eastern Finnish isolate (EF: GQ904716, Vaario et al. 2010a), and Southern Finnish isolate (SF: JF346748), which was isolated from a fruit-body collected beneath two trees (*P. sylvestris* and *P. abies*) in our study site (Fig. 1) in 2008. Cultures were maintained on modified MMN medium (Vaario et al. 2010a) with a cellophane sheet on the surface.

Field study

Sampling spots were selected on the basis of sporocarp occurrence and the presence of *T. matsutake* mycelium was confirmed by DNA extraction and subsequent polymerase chain reaction (PCR) amplification with primers specific to *T. matsutake* (Kikuchi et al. 2000) annealing at 52°C. Samples of mineral soil that yielded target PCR products were designated Shiro+and organic soil samples taken directly above as Shiro+abv. Correspondingly, matsutake-free controls (no amplification) were sampled 1–10 m to the east of Shiro+spots and designated Shiro−/Shiro-abv.

Soil sampling for hemicellulolytic enzyme assay

We randomly sampled five Shiro+and five Shiro−spots from five fruiting patch sites in 2008 (Fig. 1). A 10-ml sample of the mineral soil layer from each spot was collected after sporocarps were harvested and designated Shiro+if PCR-positive. Control spots (Shiro−, PCR negative) were sampled 1 m to the east of Shiro+spots. The five Shiro+spots were located in different sporocarp fruiting patches.

Soil sampling for organic carbon analysis

We randomly selected three sporocarps from each patch during the 2009 harvest and sampled 10 ml of mineral and 10 ml of organic soil below or next to the sporocarp, respectively. PCR-positive samples taken from the mineral layer of low-productivity patches (1 and 2) are designated as LShiro+, and those from high-productivity patches (3–5) are designated as HShiro+ (Fig. 1). Soil samples taken directly above the Shiro+spots in the organic layer are accordingly designated as LShiro+abv and HShiro+abv. Control samples were taken from a single spot 1 m to the east of a Shiro+spot in each patch and five spots were sampled 10 m to the east of the northern margin of each patch and 10 m east of the southern margin. All controls were confirmed as PCR-negative and designated Shiro−or Shiro-abv (organic soil layer). Each soil sample was immediately frozen and stored at −20°C until processing. In total, 6 sets of LShiro+/LShiro+abv, 9 sets of HShiro+/HShiro+abv, and 15 sets of Shiro−/Shiro-abv were measured.

Measurements

Enzyme activity assays

The hemicellulolytic enzyme assays were based on 4-methylumbelliferone (MU) reaction products. Substrates and the corresponding enzymes were MU-β-D-xylopyranoside (xylosidase, EC 3.2.1.37) and MU-β-D-glucuronide hydrate (glucuronidase, EC 3.2.1.31). In the Petri dish microcosm experiment, we followed the protocol of Courty et al. (2005) except that mycorrhizal root tips were replaced with five bark pieces. The dried bark pieces were weighed and results are given as per gram dry weight (gdw). The results are calculated as described in Pritsch et al. (2011) and activity is expressed as moles per gdw per minute. Soil samples were prepared according to Heinonsalo et al. (in review); briefly, 500 μl of soil was placed into a microcentrifuge tube with 500 μl sterile distilled water and centrifuged for 30 min at 16,000*g*. The supernatant was pooled from three replicate tubes and diluted to 5 ml with pure water. After mixing, the tube was stored on ice until analysis later on the same day. In contrast to Courty et al. (2005), water in the reaction mix was replaced by the soil solution.

Determination of hemicellulotic carbohydrates

Total hemicellulotic carbohydrates were determined separately for bark pieces (10–20 mg dw) used in Petri dish experiment, the liquid media (2 ml from the start and after 12 days of incubation) in the glass flask experiment, and soil samples (10–20 mg dw) taken from the field site according to Sundberg et al. (1996). The sample was exposed to acid methanolysis, which degrades non-cellulosic polysaccharides to their monomeric units. The concentration of neutral and acidic sugar units was measured by gas chromatography (GC). The concentration of total hemicellulotic compounds (hemicelluloses) was calculated as the sum of individual sugars, namely, mannose (Man), glucose (Glc), galactose (Gal), xylose

(Xyl), arabinose (Ara), and rhamnose (Rha), and of glucuronic acid (GlcA), galacturonic acid (GalA), and 4-*O*-Me-glucuronic acid (Me-glcA) including pectin. Cellulose concentration was determined by GC after acid hydrolysis and silylation according to Sundberg et al. (2003). In the microcosm experiment, total hemicelluloses for bark pieces after 20 days of culture were not measured because a certain amount was detected from matsutake hyphae (ca. 400 mg/g dw). Non-cellulosic polysaccharides in hyphae included a large portion of glucose, mainly as β-glucan, a common carbohydate in fungi (Manners et al. 1973). Hemicellulose concentration was calculated according to Merilä et al. (2010).

Statistical analyses

The impact of isolate effect on enzyme activity rates was evaluated using ANOVA; explanatory factors were isolate, time, and their interaction. Net biomass increase after 60 days of incubation in test media (different carbon source) was evaluated with Tukey's test. The effects of soil properties on productivity at the field site were evaluated using a linear mixed model analysis followed by LSD pairwise comparisons; class of mushroom productivity (low-, high-, and non-matsutake spots) was held as a fixed effect and the location of sampling spots as a random effect (seven locations including five fruiting patch sites and two control locations 10 m away). Soil layer samples were analyzed separately. Data were transformed when necessary. Normality and homogeneity of variance among residuals were examined using scatter plots and Q-Q plots. All analyses were performed with SPSS (v. 15.0; SPSS Inc., Chicago, IL, USA).

Results

Microcosm experiment

The total amount of hemicellulotic carbohydrates in autoclaved bark pieces was about 150 mg g^{-1} dw: Man (18%), Glc (11.8%), Gal (10.2%), Xyl (27.6%), Ara (14.7%), Rha (2.3%), GlcA (0.9%), GalA (13.1%), and Me-glcA (1.3%).

After 3–4 days of incubation, growing hyphae became visible at the edge of the mycelial plugs in plates containing bark pieces, and after 10 days they had reached their surface. After 20 days of incubation, growth increased and expanded over the layer of bark pieces. No isolates exhibited clear and continuous growth on glass beads. The activity rates of both enzymes were significantly higher (xylosidase, 487 mmol g^{-1} (dw bark) min^{-1}; glucuronidase, 18 mmol g^{-1} (dw bark) min^{-1}) in inoculated bark plates compared to those that had not received mycelial plugs (zero or negligible enzyme activity). Significant differences in xylosidase activity were detected among the three isolates tested: EF had a significantly higher activity rate than JA or SF (Table 2). No significant effect of time or interaction between isolate and time was found.

Liquid medium experiment

The addition of softwood or hardwood hemicellulose as the sole carbon source in a liquid medium significantly stimulated the growth of *T. matsutake* mycelia. After 60 days of incubation, a similar net increase of mycelial biomass was observed in media containing either glucose (C+) or hemicellulose (Hs or Hb) (Fig. 2a). However, limited growth was observed in carbon free (C−) and cellulose (Cel) media and in the medium containing cellulose+0.1 g/l of glucose (Celg) (Fig. 2a). Significant differences were found among isolates grown in Hs and Hb media; SF net biomass increase was 214–217% and EF 167–176% that of JA on either hemicellulose medium after 60 days of incubation (Fig. 2b). Differences in growth between EF and SF were also significant on Hs but not on Hb (Fig. 2b). No significant growth differences among isolates cultured on C+, C−, or Cel media were found (Fig. 2b).

Distribution of hemicellulotic carbohydrates in liquid media during incubation

The major hemicellulotic sugars were Man, Glu, Xyl, and Gal in both Hs and Hb media. However, there were clear proportional differences between them; most of the sugar in Hs was Man, whereas it was Xyl in Hb (Fig. 3; Leppänen et al. 2011). After 12 days of incubation, the loss of total hemicellulotic carbohydrates was significantly different among isolates. JA isolate grew less but consumed more hemicellulotic carbohydrates in 12 days than EF and SF in Hs (Fig. 4). However, JA consumed less hemicellulotic carbohydrate in Hb and its final growth was restricted (Fig. 4). On Hs medium, JA consumed mainly Man (63%), but EF and SF used only 12–13% of this sugar (Fig. 5). Although the total

Table 2 Activity rates of hemicellulolytic enzymes of bark—*T. matsutake* cultures and the significances of the isolate effect

Isolates	Xylo	Gluc
JA	440±46[a] b	25±9 a
EF	631±49 a	23±7 a
SF	526±29 ab	7±2 a

Xylo xylosidase, *Gluc* glucronidase

[a] The means [(pmol g^{-1} dw bark) min^{-1}] (± Ses) difference is significant at the 0.05 level, n=18 (LSD)

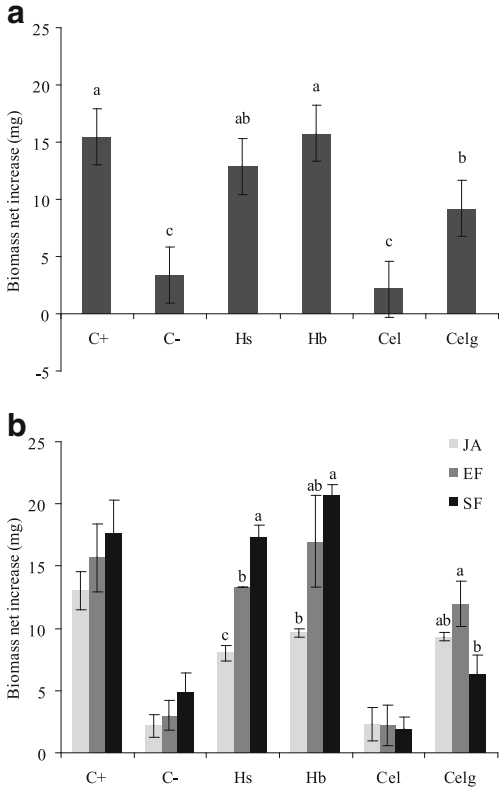

Fig. 2 Growth of *T. matsutake* after 60 days of incubation in media containing different carbon sources. Mean values and standard errors of **a** all tested isolates of *T. matsutake* (n=9). *Common letters* indicate non-significant differences among media. **b** Each isolate of *T. matsutake* (n=3). *Common letters* indicate non-significant differences among the three isolates tested (Tukey's test, $p<0.05$). *C+* carbon positive, *C−* carbon negative, *Hs* hemicellulose from spruce, *Hb* hemicellulose from birch, *Cel* cellulose, *Celg* cellulose plus a start glucose, *JA* Japanese isolate Tm0945, *EF* Finnish eastern isolate GQ904716, *SF* Finnish southern isolate JF346748

hemicellulose consumed was lower on Hb, JA used mostly Glc (90%). Similar to Hs medium, isolates EF and SF only used 22–24% Glc but were both more heavily reliant on Xyl (ca. 40%) (Fig. 5).

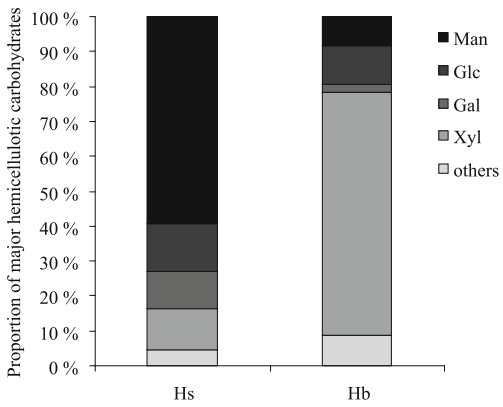

Fig. 3 Sugar unit proportions in two types of hemicellulotic carbohydrates. *Man* mannose, *Glc* glucose, *Gal* galactose, *Xyl* xylose; others including arabinose, rhamnose, glucuronic acid, galacturonic acid and 4-*O*-Me-glucuronic acid

Enzyme activity and the influence of organic matter in the shiro

Immediately after sporocarps were harvested, xylosidase activity was significantly higher in Shiro+than Shiro− ($p<0.05$), but no or negligible activity detected for glucuronidase was recorded in Shiro+ and Shiro−samples (Fig. 6). In both soil layers, high-productivity spots (HShiro+) were associated with significantly higher levels of organic matter and carbon than non-productivity spots (Shiro−). The nitrogen content of the organic soil layer was significantly higher in HShiro+spots than in Shiro−. Soil moisture content was higher in HShiro+ than others in the mineral layer, but no significant differences were present in the organic layer (Table 1). The total amount of hemicellulotic carbohydrates was significantly higher in HShiro+ than non-productivity spots (Shiro−) in both soil layers (Fig. 7). Relative proportions of hemicellulotic sugars were similar among HShiro+, LShiro+, and Shiro− in both soil layers, except that the Glc fraction in Shiro-abv (36.2%) was significantly higher than that in LShiro+abv (29.2%) and HShiro+abv (29.8%) (Fig. 7).

Discussion

Ectomycorrhizal (ECM) symbiosis is a widespread and important component of the soil ecosystem, but the fungi involved may occupy one or more positions along the

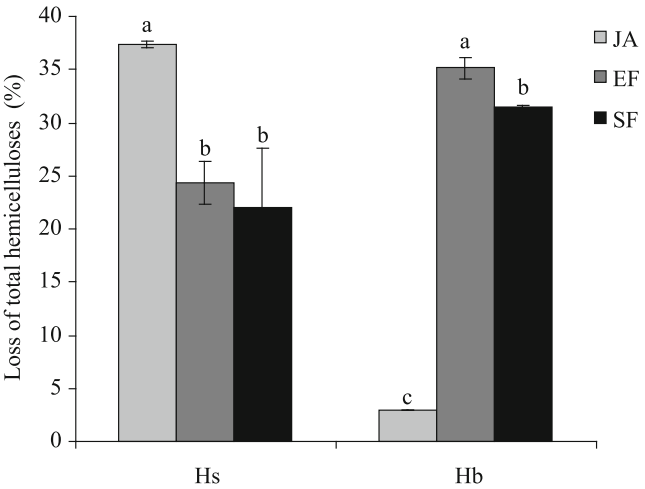

Fig. 4 Loss (%) of total hemicellulotic carbohydrates after 12 days of incubation. Mean values and standard errors of hemicellulose from spruce wood (*Hs*) and from birch wood (*Hb*). *JA* refers to Japanese isolate Tm0945, *EF* refers to Finnish eastern isolate GQ904716, and *SF* refers to Finnish southern isolate JF346748. *Common letters* indicate non-significant differences (Tukey's test, $p<0.05$, n=3)

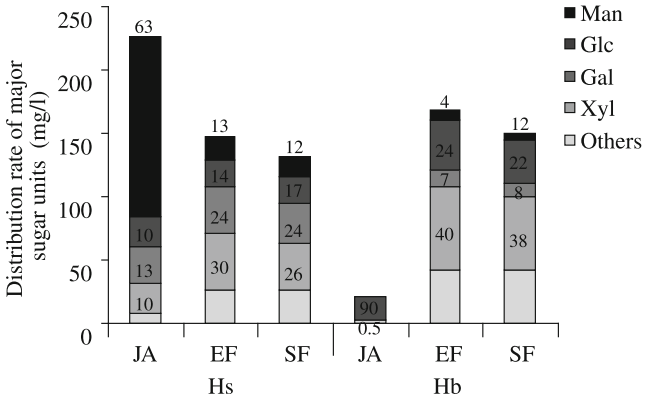

Fig. 5 Relative proportions of the main hemicellulotic sugar units in loss of total hemicellulose (mg/l) after a 12-day incubation among the three isolates tested. *Column numbers* indicate percentages of sugar unit in total hemicellulose. *JA* Japanese isolate Tm0945, *EF* Finnish eastern isolate GQ904716, *SF* Finnish southern isolate JF346748), *Hs* hemicellulose from spruce wood, *Hb* hemicellulose from birch wood, *Man* mannose, *Glc* glucose, *Gal* galactose, *Xyl* xylose; others including arabinose, rhamnose, glucuronic acid, galacturonic acid, and 4-*O*-Me-glucuronic acid

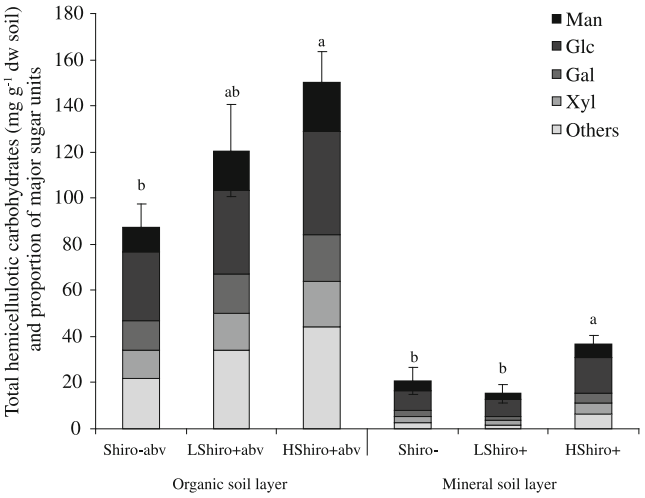

Fig. 7 Mean values and standard errors of hemicellulotic carbohydrates and relative proportions of their main sugar units in soil samples taken from the study site. *Common letters* indicate non-significant differences among three types of sample in separate soil layers (LSD, $p<0.05$, $n=6–15$). *Man* mannose, *Glc* glucose, *Gal* galactose, *Xyl* xylose; others including arabinose, rhamnose, glucuronic acid, galacturonic acid, and 4-*O*-Me-glucuronic acid

biotrophy–saprotrophy continuum (Taylor and Alexander 2005). Our study focused on the mechanism(s) by which a typical ECM fungus, *T. matsutake*, acquires carbon in vitro and yielded four key findings: (1) *T. matsutake* is able to produce hemicellulolytic enzymes in vitro, (2) *T. matsutake* can use hemicellulose as its sole carbon source in vitro, (3) enzyme activity in vivo is consistent with the observed use of hemicellulotic carbohydrates in vitro, and (4) the observed use of hemicellulose varied among three isolates of *T. matsutake*.

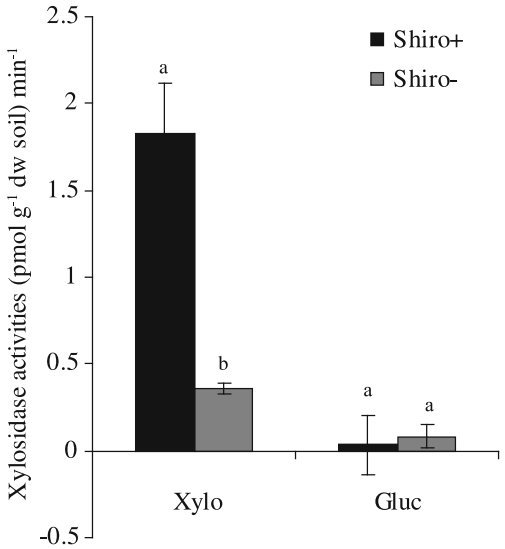

Fig. 6 Enzyme activities in soil samples dominated by *T. matsutake* (*Shiro+*) and without *T. matsutake* (Shiro−) from the study site in 2008. Mean values and standard errors of xylosidase (*Xylo*) and glucuronidase (*Gluc*). *Common letters* indicate non-significant differences between two types of soil in separate enzymes (ANOVA, $p<0.05$, $n=5$)

Results from the microcosm experiment confirmed that *T. matsutake* is able to grow on a pure bark substrate and produce hemicellulolytic enzymes appropriate to saprotrophic feeding on this organic medium. Furthermore, enzyme activities between days 20 and 40 of incubation were similar and the continued growth of hyphae during a 2-month observation period suggests that this feeding mode is sustainable.

To our knowledge, the liquid media experiments are the first to demonstrate unequivocally that *T. matsutake* can grow on a medium containing only hemicellulose as an organic carbon source. This result is convincing evidence for saprotrophic feeding in *T. matsutake.* In the field, increased activity of hemicellulolytic enzymes in Shiro+samples supports the inference from the laboratory experiments. Hemicellulose is a major component of plant material and comprises about 14.7% of hemicellulotic carbohydrates in the bark of Scots pine, the main host tree for *T. matsutake* in Fennoscandia (Valentin et al. 2009). Hemicellulose consists of several different sugar units and substituted side chains in the form of a low molecular weight linear or branched polymer. This polymer is more water soluble than cellulose with a degree of polymerization of less than 200. The most abundant hemicellulotic sugar in root bark is xylose and, according to enzyme activity measured in vitro, it serves as the primary carbon source for *T. matsutake* when grown on this medium.

Leaf litter (Park et al. 2002; Uselman et al. 2009; Hansson et al. 2010) and root exudates (Kramer et al. 2010) are considerable sources of dissolved organic carbon in soil. Hemicellulose is more soluble than cellulose and has been found in root and leaf litter in relatively high concentrations

(Kiikkilä et al. 2011). Given the results of earlier studies and that *T. matsutake* secretes hemicellulolytic enzymes and can use hemicellulose directly, we suggest three possible mechanisms to explain how it gains extra energy when symbiosis is interrupted during fruit-body formation. First, hemicellulose is a natural and abundant compound existing in the litter layer that is leached to the mineral layer where it becomes available to *T. matsutake*. Second, when the host reduces its carbon supply to the shiro, matsutake might degrade and absorb its root tissue in order to fuel extraradical mycelia while searching for healthy host roots. It has been shown that *T. matsutake* invades xylem in sawdust culture (Vaario et al. 2003) and can produce β-glucosidase on pine bark substrate (Vaario et al. 2002), an appropriate enzyme for the degradation of cellulose. Furthermore, cellobiose, a product of the enzymatic hydrolysis of cellulose, is a suitable carbon source for *T. matsutake* (Lun et al. 2004). Although plausible, the existence of this mechanism in nature has yet to be demonstrated and the isolates screened in this study failed to grow significantly in liquid culture containing cellulose as a carbon source. The occurrence of several saprotrophic and mycorrhizal/litter-decay fungi (e.g., *Trichoderma viride*) in soil above the shiro is positively correlated with *T. matsutake* (Vaario et al. 2010b). If matsutake cannot degrade cellulose directly, cellobiose and other products hydrolyzed by commensal fungi could be leached to the shiro.

Matsutake typically does not transport nutrients over long distances to form sporocarps (Ogawa 1978). Rather, the fungus usually absorbs nutrients from adjacent mycelia. The localized death of mycorrhizal root tips suggests that disconnection from the host occurs during sporocorp formation and that the energy and nutrient demands must be met solely by trophic activity of *T. matsutake*. Here, the ability to use hemicellulotic compounds directly is of profound importance. By degrading organic compounds, including its own mycelia, *T. matsutake* has the capacity to switch from a symbiotic to saprotrophic lifestyle. This is consistent with a recent review of ECM ecology in which Cullings and Courty (2009) proposed that ECM fungi occupy one or more positions along a biotrophy–saprotrophy continuum.

A link between ECM fungi and saprotrophy is perhaps uncontroversial when one considers that molecular phylogenetic, biogeographical, and palaetontological evidence imply a common origin of ECM and saprotrophic fungi. Hibbett et al. (2000) confirmed that ECM fungi have evolved repeatedly from saprotrophic ancestors and suggested that multiple reversals from ECM to the free-living condition have also taken place. Recently, they updated their new results and suggested that the ancestor of the Agaricomycetes could not have been an ECM-forming species (Hibbett and Matheny 2009). On the other hand, *Laccaria bicolor* expresses a number of enzymes in prospecting hyphae that allow it to act as a weak saprotroph, although only enough to supply a relatively small amount of carbon (Martin et al. 2008). Matsutake, as a late-stage fungus, may differ from the early-stage *L. bicolor*. In addition, GH5 cellulasese is expressed by both ECM fungi during symbiosis although the role of this enzyme is still unknown (Martin et al. 2008). The reality probably exists in a dynamic state between mutualism and parasitism, depending on environmental conditions and organism life stages. Bödeker et al. (2009) have demonstrated that several ECM fungi possess many of the genes involved that can be variably expressed in response to carbon availability and enable the switch from symbiotic to saprophytic feeding.

Notably, we found differences in the extent to which isolates used hemicellulose in artificial culture. Isolate JA (Japanese) grew poorly on hemicellulose as a sole carbon source compared to the Finnish EF and SF isolates. Furthermore, JA consumed most of mannose from Hs in the beginning of incubation (12 days), which is the main sugar unit in softwood, but was not able to use xylose in the hardwood hemicellulose (Hb). Relatively low xylosidase activity of JA may explain this. In order to incorporate these findings in artificial culture techniques, understanding variation in the metabolism of hemicellulose in *T. matsutake* would be necessary to formulate an appropriate organic substrate for the particular strain.

In conclusion, isolates of *T. matsutake* from Japan and Finland can use hemicellulose as a sole carbon source in vitro and enzyme activity suggests that this also takes place in vivo. Results linked and quantified the general relationship between enzymes secreted by *T. matsutake* and its capacity to degrade polysaccharides such as hemicellulose. It seems that *T. matsutake* can use certain organic carbon sources and/or degradation products leached to the mineral layer from soil horizons above. Thus, *T. matsutake* lives as an ECM symbiont but when necessary can also grow as a saprotroph on soil organic matter. Whether *T. matsutake* can simultaneously use carbon provided by the host and soil organic matter via its own enzyme battery remains to be investigated. There is no unequivocal evidence that any ECM fungi can complete its life cycle in the absence of a host. However, a flexible trophic ecology confers a considerable advantage to *T. matsutake*, which is now known to produce cellulolytic and hemicellulolytic enzymes in the shiro during the formation of its precious sporocarps.

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